Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested[1, 2, 3, 4]

Leslie M. Klevay,[5] *M.D., S. D. Hyg.*

Coronary heart disease is the leading cause of death in the United States (1). Of the several hypotheses developed to explain the high prevalence of this disease, that implicating the quality and quantity of dietary fat probably is accepted most widely. Other hypotheses, those of sugar intake (2), water hardness (3, 4), exercise (5), and fiber intake (6) have received less acceptance. Little effort has been expended in attempts to reconcile these apparently conflicting hypotheses, except to say that the disease is of multifactorial origin.

The concentration of cholesterol in human serum (7) and plasma (8) has great epidemiological importance in the prediction of risk of coronary or ischemic heart disease. Because of this predictive utility, agents under consideration for implication in the etiology of atherogenesis might be expected to affect plasma cholesterol concentration. These experiments were designed to test the hypothesis that an alteration in the ratios of metallic elements ingested by rats would alter the concentration of cholesterol in the plasma of the animals. An increase in the ratio of zinc to copper ingested by rats consuming a diet based upon sucrose, egg white protein, and corn oil, and containing no cholesterol or cholic acid produced an increase in the concentration of cholesterol. These results represent a successful test of the hypothesis and may provide a means of unifying the four hypotheses of atherogenesis mentioned.

Materials and methods

The experiments are identified by the year in which they were begun. Male weanling rats of the Carworth strain (Carworth Division, Becton Dickinson Co., New York, N. Y.) were maintained under clean conditions as previously described by Klevay et al. (9) or with identical equipment in a conventional animal room. The animals were housed in individual, stainless steel cages equipped with water bottles of soft glass with stainless steel spouts and silicone stoppers (Ronsil Division, Rodhelm Reiss, Inc., Belle Meade, New Jersey). All equipment for mixing the diets was made of stainless steel. Upon receipt, the animals were fed the purified diet shown in Table 1 and water (distilled in glass vessels) for 3 days to accustom them to their new environment and to insure uniform hydration. They were then divided into groups matched by mean weight to differences of less than 0.5 g, and the drinking solutions of zinc and copper salts were substituted for the distilled water. The purified diets and drinking solutions were supplied ad libitum. In each experiment at least one group was fed a powdered, commercial laboratory feed (Ralston Purina Co., St. Louis, Missouri) and was given distilled water to drink.

The concentrations of zinc and copper in the purified diet were generally less than 2 and 1 μg/g, respectively, amounts insufficient to permit normal growth and synthesis of hemoglobin. The animals received supplementary amounts of zinc and copper as solutions of reagent grade $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ (J. T. Baker, Phillipsburg, New Jersey) and $CuSO_4 \cdot 5H_2O$ (Fisher Scientific Co., Pittsburgh, Pennsylvania) in water distilled in glass vessels. The concentrations of zinc and copper in the control solution were 10 and 2 μg/ml, respectively. The concentrations of zinc and copper in the experimental solutions ranged from 10 to 20 and 0.25 to 0.5 μg/ml, respectively. The ratio of the weights of zinc to copper in the experimental solutions was always 40, in contrast to a ratio of 5 in the control solution. Preliminary experiments with adult rats failed to reveal any difference in water

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[4] Address reprint requests to: Dr. L. M. Klevay, USDA Human Nutrition Laboratory, P.O. Box D-University Station, Grand Forks, North Dakota 58201.

[5] Formerly, Associate Professor of Environmental Health. Presently, Research Medical Officer, Human Nutrition Laboratory.

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TABLE 1
Composition of the diet

	g
Sucrose[a]	623.5
Fibrous cellulose powder[b]	30.0
Zn- and Cu-free Jones Foster salt mix[c]	40.0
Choline chloride[d]	1.5
Egg white[e]	200.0
Rat vitamin mix[f]	5.0
Vitamin ADE mix[g]	10.0
Corn oil[h]	90.0

[a] Jack Frost, National Sugar Refining Co., Philadelphia, Pa. [b] Whatman CFll, W & R Balston, Ltd., London, England. [c] Specially prepared by Nutritional Biochemicals Corp., Cleveland, Ohio, according to (57). $ZnCl_2$ and $CuSo_4 \cdot 5H_2O$ were omitted. [d] Sigma Chemical Company, St. Louis, Missouri. [e] General Biochemicals, Inc., Chagrin Falls, Ohio. [f] The vitamin mix consists of the following components in milligrams: thiamin hydrochloride, 400; riboflavin, 800; pyridoxine hydrochloride, 400; calcium pantothenate, 2,500; niacinamide, 4,000; folic acid, 100; menadione, 10; biotin, 20; cyancobalamin, 15. These were mixed with 500 g sucrose. [g] The vitamin ADE mix had the following composition: calciferol, 5.70 mg; α-tocopherol, 10 g; vitamin A palmitate, 2 g; mixed with 1,000 g corn oil. [h] Mazola Corn Oil, Corn Products Co., Rahway, New Jersey.

consumption between experimental and control groups.

Animals were weighed weekly. Blood was collected from the tail vein into heparinized microhematocrit tubes except for the first collection in 1970 when blood was collected from the orbital plexus. Hematocrits were obtained by centrifugation to a constant value; then the tubes were broken at the interface and plasma was measured for lipid determination. Plasma cholesterol was determined by the method of Carpenter et al. (10) using both re-crystallized cholesterol and, in 1971 and 1972, a commercially available, simulated plasma (Dade Division, American Hospital Supply Corp., Miami, Florida) as standards. Measurements were done in duplicate, except for the first collection in 1970. Phospholipids were measured by the method of Chen et al. (11). Statistical analyses were done by the variance ratio test and Student's *t* test according to Snedecor (12).

The only variations among the experiments were those of experiment duration and the timing of the collection of blood samples. These parameters were varied to determine how soon the hypercholesterolemia developed and to follow its progression.

Results

Graphs of mean group weight versus age formed a series of nearly parallel curves showing differences that persisted for long periods of time. The weights shown in Table 2 are selected from the data obtained weekly and are those obtained nearest the 60th day of each experiment. Data within an experiment are comparable, but data between experiments are not. Generally rats in the experimental groups ingesting water with a ratio of zinc to copper of 40 grew less rapidly than those in the control groups ingesting water with a ratio of 5. In 1972, the first two clean groups differed significantly by the variance ratio test at 60 days, but as the significance was lost by the following week, no probability value is shown in Table 2. Growth of groups fed the purified diet was 66 to 84% of that of groups fed the commercial diet (Ralston Purina Co.).

The reason for the slower growth of rats in the experimental groups compared with control

TABLE 2
Weights of rats after approximately 60 days[a]

Experiment[b]	Zn/Cu[c]	Body weight, g[d]	Body weight, %[e]
1970			
Clean	10/2	282 ± 4.8	84.3
	20/0.5	249 ± 7.0$^{0.001}$	74.3
1971			
Clean	10/2	260 ± 7.7	75.4
	10/0.25	229 ± 16.2$^{0.050}$	66.4
Conventional	10/2	293 ± 9.1	83.3
	10/0.25	252 ± 7.9$^{0.005}$	71.6
1972			
Clean	10/2	248 ± 6.0	
	10/0.25	242 ± 12.4	
	20/0.5	233 ± 7.4	
Conventional	10/2	262 ± 11.7	74.2
	20/0.5	233 ± 10.1	66.0

[a] Nine rats/group except in 1970 when 18 rats were used. [b] Clean refers to rats raised under the conditions described in (9). Conventional refers to rats raised by using identical equipment, but in ordinary animal quarters. Intestinal flora are assumed to be similar in either case. [c] These are the concentrations in micrograms/milliliter of Zn and Cu in the drinking water. [d] Mean ± SEM; superscripts indicate the maximum probability value obtained in an F or *t* test in comparison with the Zn/Cu = 5 group raised under comparable conditions. Absence of a superscript indicates $P > 0.05$. The *t* tests were done if no heterogeneity of variance could be demonstrated with the F test. [e] Expressed as percent of the weights of rats fed powdered Laboratory Chow, for rats, mice, and hamsters formulated by Ralston Purina Co., St. Louis, Mo.

groups is inapparent. It cannot be attributed to toxicity of zinc because preliminary experiments with adult rats demonstrated that the intake of zinc of those consuming water containing 20 μg Zn/ml was approximately one-third that of rats consuming the commercial diet. It cannot be attributed to zinc deficiency, as rats consuming water containing 20 μg Zn/ml did not grow more rapidly than did rats consuming water containing 10 μg Zn/ml.

Data on hematocrits are shown in Table 3; anemia was found in all experimental groups.

The concentration of phospholipids in plasma was determined twice in 1970. No significant difference was found on the 84th day; however, by the 262nd day, concentrations of 274 ± 20.6 and 385 ± 35.5 mg/100 ml (mean ± SEM, $P < 0.01$) were found for the control and experimental groups, respectively.

The data on the concentrations of cholesterol in plasma are shown in Table 4. Analyses were done several times during each experiment; the tabulated data are those first found to be significantly different. Usually the probability values associated with the differences decreased at subsequent times indicating that the difference between experimental and control groups was increasing. For example, in 1970 the probability values on the 120th, 196th, and 254th days were less than 0.025, 0.005, and 0.005, respectively. In 1971, the probability for the clean group was less than 0.005 on the 82nd, 151st, 219th, and 270th days, after which time death reduced the number of animals available for study. For the conventional group statistical significance was lost on the 219th day, but the values for the experimental group still exceeded those for the control group on the 270th day. No samples were collected for analysis in these two experiments earlier than the 84th or 45th days, respectively. The decreasing probability values with time indicate that the hypercholesterolemia had not completely developed at the time statistical significance was first demonstrable.

In 1972, samples were analyzed at approximately monthly intervals to determine the time of earliest demonstrable difference. In this experiment, in contrast to that of 1971, the first demonstrable difference was found in the conventional group. Among the clean groups, the one receiving 0.25 μg Cu/ml differed significantly from the control group on the

TABLE 3
Hematocrits of rats[a]

Experiment[b]	Zn/Cu[b]	Hematocrit, %[c]	Day[d]
1970			
Clean	10/2	55 ± 1.0	84
	20/0.5	$51 \pm 1.4^{0.050}$	
1971			
Clean	10/2	53 ± 1.2	45
	10/0.25	$43 \pm 1.9^{0.001}$	
Conventional	10/2	48 ± 0.3	45
	10/0.25	$42 \pm 1.3^{0.001}$	
1972			
Clean	10/2	53 ± 0.9	65
	10/0.25	$50 \pm 0.8^{0.025}$	
	20/0.5	$49 \pm 1.0^{0.010}$	
Conventional	10/2	50 ± 0.4	36
	20/0.5	$46 \pm 0.7^{0.001}$	

[a] Nine rats/group except in 1970 when 18 rats were used, and in 1971 when loss of two samples resulted in 8 rats in the high ratio groups. [b] See footnotes b and c in Table 2 for explanation. [c] See footnote d in Table 2 for explanation. [d] The experimental day on which significant differences were first demonstrated.

TABLE 4
Concentration of cholesterol in plasma of rats[a]

Experiment[b]	Zn/Cu[b]	Cholesterol concentration, mg/100 ml[c]	Day[d]
1970			
Clean	10/2	168 ± 15.2	84
	20/0.5	$223 \pm 20.3^{0.050}$	
1971			
Clean	10/2	94 ± 8.3	45
	10/0.25	$111 \pm 4.0^{0.050}$	
Conventional	10/2	141 ± 10.9	151
	10/0.25	$180 \pm 29.5^{0.010}$	
1972			
Clean	10/2	109 ± 3.1	93
	10/0.25	$125 \pm 3.3^{0.005}$	
	10/2	96 ± 3.8	127
	20/0.5	$118 \pm 4.5^{0.005}$	
Conventional	10/2	95 ± 3.4	37
	20/0.5	$107 \pm 3.8^{0.050}$	

[a] Nine rats/group except in 1970 when 18 rats were used. [b] See footnotes b and c in Table 2 for explanation. [c] See footnote d in Table 2 for explanation. [d] The experimental day on which significant differences were first demonstrated.

93rd day; the group receiving 0.50 μg Cu/ml differed significantly on the 127th day. These data suggest that although the ratio of zinc to copper is important in determining the concentration of cholesterol, higher absolute amounts of copper increase the time needed for hypercholesterolemia to occur.

The variance ratio and *t* tests have shown that such differences would occur only infrequently due to chance. Examination of the data obtained in these experiments before statistical significance was demonstrated reveals that the direction of the differences between groups becomes manifest much sooner. For example, in 1971 the mean concentration of cholesterol for the conventional experimental group exceeded that of the control group on the 45th day of the experiment, although statistical significance was not demonstrable until the 151st day. Similarly, in 1972, the concentration of cholesterol for the clean, experimental groups exceeded that of the control group on the 37th day, although statistical significance was not demonstrable until later.

If only the direction rather than the magnitude of the differences in concentration of cholesterol between groups is considered, another type of statistical test (13) can be used. This experiment has been done five times, three times under clean conditions and twice under conventional, environmental conditions. In each case the mean concentration of cholesterol of the experimental groups exceeded that of the control groups. The probability of such uniformity of result is one-half raised to the fifth power, or 0.031 (13).

Comparison of the data for 1971 and 1972 in Tables 3 and 4 reveals that changes in concentration of cholesterol generally occurred after the changes in hematocrit. In 1972, changes in concentration of cholesterol occurred before changes in body weight occurred. Blood samples were not taken early enough to establish such a relationship in the earlier experiments.

In these experiments all the rats were of the same strain and were obtained from the same source. All dietary materials also came from the same sources. Both rats and diets may be presumed to have varied slightly in a period of 3 years. Such variations did not reduce the consistency with which a ratio of zinc to copper of 40 produced an increase in the concentrations of cholesterol compared with a ratio of 5 in two different environments.

In 1972, the mean concentration of cholesterol for the control group raised under clean conditions was significantly lower on the 127th day than on the 93rd day ($P < 0.025$). Statistical significance was lost if the values for the 2 days were adjusted to compensate for the slight differences found for the concentration of cholesterol in the standard, simulated plasma on those 2 days. Thus, the difference between the control groups on those 2 days represents an analytical artifact. At no time during any of the experiments did the concentration of cholesterol determined in the simulated plasma differ from the true value by more than 10%, and usually the difference was much less than 10%.

Although the experimental groups had concentrations of cholesterol that consistently exceeded those of the control groups at each analysis, the values for the control groups varied considerably when the several experiments were compared. For example, the concentrations for the clean control groups ranged from 94 to 168 mg/100 ml in 1971 and 1970, respectively. These differences and the hypercholesterolemia of some of the control groups cannot be attributed to analytical error because of the consistency with which the values determined on the simulated plasma resembled the true values. These differences are probably due to some aspect of the experimental situation that remains to be identified.

Discussion

Coronary heart disease is probably the result of a slow, progressive, complicated process, the nature of which is not understood. The hypothesis relating the quality and quantity of dietary fat consumed to the risk of coronary heart disease has achieved wide acceptance; dietary fat may represent the largest single factor in the genesis of this disease. It is hypothesized that the four "other" factors mentioned increase the ratio of zinc to copper available for absorption from the intestinal tract, increase the ratio of zinc to copper retained within the body following absorption, or alter the distribution of these elements in certain important organs resulting in an increase

of the ratio of zinc to copper. This increase in the ratio of zinc to copper results in an increase in the concentration of cholesterol in plasma and, presumably, an increase in the risk of coronary heart disease. Evidence in support of this hypothesis is based upon observations on populations, individuals, and animals.

Evidence based upon observations of populations

Yudkin (2) and Trowell (6) have suggested that ischemic heart disease is associated with a high consumption of sugar (2) and low consumption of vegetable fiber (6) in those countries where the disease is prevalent. These two hypotheses are related because diets containing large amounts of refined sugar are likely to contain small amounts of vegetable fiber and vice versa.

Yudkin (14) states that there is a one to one (weight basis) relationship between the amount of fat and sugar consumed in the 41 countries for which data are available. As there is a physiological limit upon the amount of food that can be consumed regularly, those diets containing large amounts of fat and sugar must of necessity contain small amounts of unrefined carbohydrate. Decreased consumption of unrefined carbohydrate, for example, cereals, nuts, and legumes, results in decreased consumption of phytic acid,[6] as Averill and King (15) and McCance and Widdowson (16) have shown phytic acid to be closely associated with these sources of carbohydrate.

Cereals, nuts, and legumes, the foods shown to contain phytic acid, also contain fibers (6). Thus, diets that are high in sugar are also low in fiber and phytic acid; these are the diets associated with high risk of ischemic heart disease.

These data on populations can be related to the present hypothesis only upon consideration of data from experiments with animals. Oberleas et al. (17) have shown that phytic acid reduces the availability of dietary zinc. No data on the availability of dietary copper were presented. The work in vitro of Vohra et al. (18) shows that at an alkaline pH similar to that of the small intestine where these two metallic cations (19, 20) are absorbed, the copper complex with sodium phytate is soluble, whereas the zinc complex is not. Thus, phytic acid may have the protective effect of reducing the ratio of zinc to copper available for absorption.

Several reports (3, 4) have demonstrated that residence in areas where hard water is drunk is associated with a lower risk of coronary heart disease than is residence in areas with soft water. There is such a close relationship between hardness of water and the concentration of calcium in the water that in the reports of the United States Geological Survey, hardness of water is reported as calcium carbonate (21). Crawford et al. (22) state that the correlation coefficient between hardness and calcium equals 0.95. They (22) also state that although the concentration of zinc was similar in hard and soft waters studied, the mean concentration of copper was higher in the hard waters. These data on the effect of hard water on risk of populations can be related to the present hypothesis upon consideration of experiments with animals.

According to Guggenheim (23), "meat anemia" in mice is the result of an insufficient amount of dietary copper in the presence of an excessive amount of dietary zinc. The disease is accentuated by a concomitant lack of calcium, and can be prevented by the addition of calcium to the diet (24). Heth et al. (25) found that calcium added to the diets of rats resulted in a shift of zinc from the liver to bone even when no change in absorption of zinc occurred. This interaction among calcium, copper, and zinc (23–25) is apparently independent of the absorptive interaction among calcium, zinc, and phytic acid (17).

Thus, the drinking of hard water may exert a protective effect in two ways. The concentration of calcium in hard water is such (21) that the calcium ingested via drinking water may be as high as 22% of the recommended daily allowance (26) or 40% of the suggested practical allowance (27). This increased consumption of calcium may cause a change in the distribution of zinc within the body resulting in a decrease in the ratio of zinc to copper in the liver, the major site of cholesterol synthesis (28). The higher concentration of copper in hard water would decrease this ratio and that of the zinc to copper absorbed from the intestinal tract directly.

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[6] Phytic acid is the trivial name for *myo*-inositol hexaphosphate (European J. Biochem. 5: 1, 1968).

Men who exercise regularly have a lower risk of coronary heart disease than men who do not exercise (5). The concentrations of zinc and copper in sweat have been determined by Prasad et al. (29) and Mitchell and Hamilton (30) to be 0.93 and 0.058 μg/100 ml, respectively, the ratio of zinc to copper being approximately 16. Thus, an increase in physical activity leading to an increase in sweating may have the protective effect of causing a relatively greater loss of zinc than of copper from amounts already absorbed and available for metabolic use, resulting in a lower ratio of zinc to copper.

Evidence based upon observations of human individuals and animals

Some chelating agents have been shown to alter the concentration of cholesterol in plasma. Perry and Perry (31) treated eight hypercholesterolemic patients with calcium disodium ethylenediaminetetraacetate. The mean decrease in the concentration of cholesterol in plasma was 96 mg/100 ml. Contemporaneously the concentration of zinc in urine rose elevenfold. No data on the change in the concentration of copper in urine were included in this article; however, the authors implied the increase in copper concentration was of similar magnitude to that found in a single patient reported earlier (32), approximately 5%. That patient experienced a decrease in the concentration of cholesterol of 107 mg/100 ml. The disproportionately high loss of zinc compared with that of copper occurring as the concentration of cholesterol in plasma was decreasing might be considered analogous to the effect of sweating mentioned previously. The greater loss of zinc results in a decreased ratio of zinc to copper resulting in a lower concentration of cholesterol in plasma.

Histidine is an amino acid with chelating properties almost ideally suited for testing the hypothesis that an increase in the ratio of zinc to copper can cause an increase in the concentration of cholesterol in plasma. Maley and Mellor (33) and Hallman et al. (34) found the ratio of the formation or stability constants between histidine and the dispositive ions of copper or zinc ranged from 3,020 to 651,000, depending upon the definition used and the conditions of measurement. It seems likely that with an affinity for copper several thousand times as great as for zinc, histidine might well alter the ratio of zinc to copper in biological systems.

During normal human pregnancy the concentrations of cholesterol and phospholipids in serum increase by about 40% (35). Less well-known is the histidinuria that occurs late in pregnancy. Page et al. (36, 37) have attributed this loss of histidine to increased renal clearance. No data were found regarding relative urinary losses of zinc and copper during pregnancy. Probably no one has measured these ions in urine as the major excretory pathway for both of them is via the feces (38).

Histidinemia is a rare, hereditary, metabolic disorder characterized by an inability to catabolize histidine resulting in increased concentrations of histidine in blood and urine (39). Approximately 50 cases have been identified; so far, only a single measurement of cholesterol has been reported in one case. Ghadimi et al. (40) examined a 3-year-old girl and found the concentration of cholesterol in her serum to be 190 mg/100 ml. The histidinemic child (40) had been consuming a diet low in phenylalanine at the time cholesterol was measured because of an initial diagnosis of phenylketonuria. She may have been consuming a diet high in protein and low in fat at the time of her admission to the hospital. Diets low in phenylalanine tend to be low in fat (41). If she was consuming a commercially prepared diet (Lofenalac, Mead Johnson Co., Evansville, Indiana) widely used in the treatment of phenylketonuria, that diet was free of cholesterol, was low in fat, and contained corn oil as the only fat (42). It is likely that the diet also contained sitosterol (43). Such diets reduce the concentration of cholesterol in serum (43).

Drash (44) has reviewed the concentration of cholesterol found in serum early in childhood. Mean concentrations of 64, 159, and 165 mg/100 ml were found at birth, between 1 and 2 years, and between 2 and 6 years, respectively. These values were determined for normal children consuming normal diets. Consequently, it is possible to interpret a single measurement of 190 mg/100 ml on the histidinemic child (40) as representing hypercholesterolemia under these circumstances.

The observations of hypercholesterolemia in normal pregnancy and in a single case of histidinemia are consonant with an increased

urinary excretion of histidine causing a disproportionately large loss of copper compared with zinc. This loss then causes an increase in the ratio of zinc to copper in the body which, in turn, causes an increase in the concentration of cholesterol in plasma.

Geison and Waisman (45) produced an increase in the concentration of cholesterol in plasma of rabbits by feeding them excess histidine. Previous experiments with monkeys and rats were said to have had similar results. The inability of Woodworth and Baldridge (46) to produce an increase in the concentration of cholesterol by feeding histidine to rats may be explained by their use of a purified diet with a salt mix having a ratio of zinc to copper of 1.57 (47).

The unsuccessful attempt of Gerber et al. (48) to produce hypercholesterolemia in human subjects by feeding histidine may be explained by the biologically insignificant increase in the concentration of histidine in the serum of their subjects. According to the normal data of La Du (39), this increase was approximately 30%. In contrast, Geison and Waisman, who were successful in experiments with animals, produced a severalfold increase (45).

The concentration of 17β-estradiol in plasma increases sixfold (35) in normal human pregnancy. Evans et al. (49) have shown in rats that this compound causes a decrease in the concentration of copper in liver. No data on the concentration of zinc were published. These data supplement those on histidinuria, and are consonant with an increase in the ratio of zinc to copper in the liver, the major site of cholesterol synthesis (28). This change in the distribution of copper within the body may contribute to the hypercholesterolemia of pregnancy.

Wester (50) measured 26 trace elements in the uninjured, presumably healthy, myocardial tissue of victims of myocardial infarction and the myocardial tissue of the victims of accidents. No significant difference was found in the concentration of zinc between the two groups; however, the concentration of copper was lower in the tissue from hearts with infarctions. These data are consonant with the hypothesis that an increase in the ratio of zinc to copper produces hypercholesterolemia and death from coronary heart disease.

Few studies have been found in which total, daily intakes of both zinc and copper have been estimated. The data of Schroeder et al. (51, 52), which seem to have been obtained from the same samples of an institutional diet, can be used to calculate a ratio of zinc to copper of 5. The data of White (53) yield ratios of 24 and 35 for the diets of college women and high school girls, respectively. Calculations based on the data of Price et al. (54) for a diet representative of that consumed by low income girls in the southeastern United States give a ratio of 3.

Results from preliminary experiments with adult rats indicate that the ratios of zinc to copper consumed by the control and experimental groups reported here were 4 and 17, respectively, if zinc and copper contributed by both the diet and the drinking solutions are considered. As previously noted, the ratio of zinc to copper of the purified diet was approximately 2.

The information collected by Sandstead (55) and Evans (56) on the variable availability of dietary zinc and copper and the work previously cited (17, 23–25) make necessary cautious interpretation of analytical data on human diets. However, comparison of the ratios of zinc to copper of human diets with the ratios of intakes of the rats reported here under conditions of invariable availability indicate that although some humans consume diets comparable in ratio to the control animals, others consume diets with ratios exceeding those of the experimental animals. Thus, published data suggest that the dietary conditions of these experiments resemble those of human diets closely enough to support the belief that these experiments are of epidemiological importance.

Summary

The experiments were designed to test the hypothesis that an alteration of the amount of metallic elements ingested by rats would produce a change in the concentration of cholesterol in the plasma of the animals. Rats were fed ad libitum a purified diet based upon sucrose, egg white protein, and corn oil, and containing no cholesterol or cholic acid; intakes of zinc and copper were varied by varying the ratios of salts of these elements in the drinking

water. Drinking solutions were made with analytical grade reagents dissolved in water distilled from glass vessels. The hypothesis was tested successfully as over a period of 3 years, in two different environments, drinking water with a ratio of zinc to copper of 40 consistently and significantly produced higher concentrations of cholesterol in plasma than did water with a ratio of 5.

Explanations of the etiology of coronary or ischemic heart disease other than that relating risk to the quality and quantity of dietary fat consumed have not achieved wide acceptance. Data are cited supporting the hypothesis that increased consumption of sugar, decreased consumption of vegetable fiber, consumption of soft water, and lack of exercise result in an increase of the ratio of zinc to copper available for absorption from the intestinal tract, an increase in the ratio of zinc to copper retained in the body following absorption, or an alteration in the distribution of these elements in certain important organs. This increased ratio of zinc to copper then causes an increased concentration of cholesterol in plasma, and presumably, results in increased risk of coronary heart disease. Such increased risk may add to genetic, dietary, and other factors that influence the atherogenic process(es).

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